Journal of Medical Virology 48:129–132 (1996)

Polymerase Chain Reaction in the Investigation of "Relapse" Following Herpes Simplex Encephalitis

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Five cases of apparent relapse of herpes encephalitis were investigated. All patients recovered after antiviral and corticosteroid therapy. Samples of CSF taken from the patients at intervals through the initial and subsequent encephalitic episode were examined. PCR amplification of a 351 bp sequence from the Herpesvirus simplex (HSV) thymidine kinase gene demonstrated the presence of HSV DNA in CSF taken during the initial encephalitic illness but not during the second encephalitic episode. Intrathecal synthesis of HSV antibody (HSV antibody index > 1.9) was observed in all cases following the first episode, and there appeared to be no significant increase in intrathecal antibody synthesis in the second episode. High levels of CSF myelin basic protein were found during the acute phases of both the initial and the subsequent encephalitic illnesses. These data suggest that at least in our series of five patients, relapse following HSE may not be due to active viral replication.

KEY WORDS: polymerase chain reaction, herpesvirus simplex, relapse

INTRODUCTION

Clinical relapse of encephalitic illness is an occasional sequel of treatment of herpes simplex encephalitis (HSE) with either vidarabine or acyclovir [Whitley et al., 1988a]. The differential diagnosis of the secondary encephalitic episode includes incomplete treatment of the initial herpetic encephalitis [Nicolaidou et al., 1993], encephalitis caused by a drug-resistant strain of herpes simplex virus (HSV) [van Landingham et al., 1988], a postinfectious encephalopathy [Koenig et al., 1979], or encephalitic illness of other aetiology. However, although the aetiology of the secondary episode of encephalitic illness in patients following treatment and apparent recovery from HSE is often uncertain, a precise definition of the cause of relapse in an individual patient is important. The selection of an appropriate therapy for the relapse and the correct choice of therapy may have profound implications in terms of subsequent morbidity.

A series of retrospective studies have suggested that detection of HSV DNA in cerebrospinal fluid (CSF) via the polymerase chain reaction (PCR) provides specific and early diagnosis of acute herpes encephalitis [Klapper et al., 1990; Puchhammer-Stockl et al., 1990; Aurelius et al., 1991; Espy et al., 1993]. Using PCR, HSV DNA can be detected as early as day 1 or 2 of neurological illness [Puchhammer-Stockl et al., 1990; Dennett, 1993]. Duration of detection of viral DNA in lumbar CSF is variable, but negative results can be obtained after only 8 or 9 days of antiviral therapy [Klapper et al., 1990; Aurelius et al., 1991; Ando et al., 1993]. We investigated five cases of apparent relapse of HSE after antiviral therapy of the initial illness. All patients recovered. Samples of CSF taken from the patients at intervals through the initial encephalitic episode and the subsequent clinical relapse were investigated for the presence of HSV DNA and as a marker of intra-CNS demyelination, myelin basic protein.

MATERIALS AND METHODS

Patient Details

Details of the five patients included in this study are shown in Table I. Patients 1, 2, 4, and 5 were treated with acyclovir for 10 days; patient 3 received vidarabine for 10 days. In each case, antiviral therapy was commenced within 5 days of the onset of neurological illness. Diagnosis of the acute episode of encephalitis as HSE was made by detection of specific intrathecal HSV antibody synthesis as previously described [Klapper et al., 1981]. CSF samples had been stored at −40°C before PCR and myelin basic protein assay.

Polymerase Chain Reaction (PCR)

Amplification of a 351bp fragment of the herpes simplex virus thymidine kinase gene was performed as previously described [Klapper et al., 1990]. Briefly, DNA was purified from 50 μl of CSF, taken during the acute and recurrent episodes of illness, by phenol/chloroform extraction and ethanol precipitation [Den-

Accepted for publication August 9, 1995.

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TABLE I. Results of CSF Analyses

Case (sex/age)	Day after onset	PCR	MBP (ng/ml)	Antibody index	IgG index
1	7	+	>320	1.92	0.58
(M/67 years)	13	+	0	Not tested	Not tested
	56[a]	−	40	6.46	3.75
2	7	+	>320	0.41	0.41
(M/16 years)	18	+	0	3.25	1.64
	52[a]	−	90	1.85	1.37
3	10	+	105	6.66	0.91
(M/57 years)	24	−	0	47.3	0.56
	86[a]	−	88	20.79	0.90
4	64[a]	−	0	7.27	1.10
(F/26 years)	75	−	0	6.40	0.70
	90[a]	−	24	6.98	0.83
5	19	+	62	7.80	1.30
(M/23 years)	70[a]	−	37	6.20	0.90
"Normal" values			<5	<1.91	<0.70

[a]Recurrences.

nett et al., 1991]. Precipitated DNA was resuspended in 20 μl sterile distilled water and 10 μl added to a PCR reaction mixture comprising; 0.01 M Tris-HC1 pH 8.3; 0.05 M KC1; 2m M $MgCl_2$; 0.02% (w/v) bovine serum albumin; 100 nM oligonucleotide primers; 200 μM deoxynucleotides; 2.5 U Taq DNA polymerase (Amplitaq, Perkin Elmer). Samples were subjected to 50 cycles of 94°C/2 minutes, 50°C/1.5 minutes, 70°C/2 minutes after an initial denaturation for 7 minutes at 94°C. Amplification products were analysed by agarose gel electrophoresis (1.5% agarose in TAE run at 5V/cm). Results were confirmed by Southern blot hybridization with a biotinylated probe [Klapper et al., 1990] homologous to a 250bp region within the 351bp amplicon.

Myelin Basic Protein Assay

Myelin basic protein in CSF samples was determined by competitive radioimmunoassay (Diagnostic Systems Laboratories, Houston, TX, catalogue no. DSL 1500).

Intrathecal Antibody Synthesis

Paired serum and CSF samples from each episode were assayed. Total IgG and albumin levels were determined by electroimmunoassay [Klapper et al., 1981]; HSV-specific IgG antibody was measured by radioimmunosorbent assay [Klapper et al., 1981]. Indices were calculated as follows:

$$\text{Antibody} = \frac{\text{CSF HSV antibody}}{\text{Serum HSV antibody}} \div \frac{\text{CSF albumin}}{\text{Serum albumin}}$$

[Klapper et al., 1981].

$$\text{IgG Index} = \frac{\text{CSF IgG}}{\text{Serum IgG}} \div \frac{\text{CSF albumin}}{\text{Serum albumin}}$$

[Delpech and Lichtblau, 1972].

RESULTS

Five cases of apparent "relapse" of encephalitis following acute herpes encephalitis were investigated. In addition to detection of HSV DNA by PCR, the quantity of myelin basic protein in CSF was measured as an illustration of the degree of demyelination occurring during each episode. Results are shown in Table I.

In each case, increased levels of myelin basic protein were noted in both the initial episode and the "relapse." The highest levels of CSF myelin basic protein were detected during the acute episode of herpes encephalitis. Following antiviral therapy and clinical recovery, myelin basic protein could not be detected in CSF. During the clinical relapse of encephalitis, myelin basic protein was again detected in CSF. This was associated with an increase in IgG Index (measuring total intrathecal IgG synthesis), but, and allowing that only limited numbers of specimens were available for analysis in each case, without a rise in Antibody Index (measuring HSV specific intrathecal IgG synthesis). HSV DNA (HSV type 1) [Dennett et al., submitted] was detected in all samples taken during the initial episode but in none of those taken during the time of apparent relapse (Table I).

DISCUSSION

The aetiology of the secondary episode of encephalitic illness in patients following treatment and apparent recovery from HSE remains unclear. Whereas the use of terms such as "relapse" or "recurrence" may seen appropriate in description of these episodes, the underlying pathogenetic mechanisms (whether viral, immune, or both viral and immune activation) are not understood, and there is no adequate definition of these terms with respect to HSV infection of the CNS.

Several explanations have been advanced in explanation of the observations in individual patients including: treatment failure through incomplete inactivation of viral replication [Nicolaidou et al., 1993]; drug resistance [van Landingham et al., 1988]; a postinfectious encephalopathy, characterised by leucocyte infiltration of infected tissue [Koenig et al., 1979], or a secondary encephalitic episode of unknown aetiology.

Where relapse has been ascribed to "treatment failure" [Knezevic et al., 1983; Rothman et al., 1988; Kimura et al., 1992; Nicolaidou et al., 1993]; the episode appears to have occurred within 2 weeks of cessation of up to 10 days of apparently successful antiviral therapy, and in such patients herpes simplex virus or its DNA has been found in either brain tissue or CSF [Knezevic et al., 1983; Rothman et al., 1988; Kimura et al., 1992; Nicholaidou et al., 1993]. This has led to the suggestion that administration of acyclovir for 10 days may be inadequate to ensure uncomplicated recovery from HSE [Quintiliani et al., 1991] and that in proven cases of HSE prolonged intravenous acyclovir therapy should be considered. The finding of long-term persistent intrathecal cellular and humoral immune activation in HSE [Aurelius et al., 1993] lends support to this suggestion. In addition, and developing from in vitro studies that have indicated synergism in antiviral activity between interferon and acyclovir, combination therapy for acyclovir with either α or β interferon has been attempted and suggested as being more effective in prevention of serious sequelae of HSE [Prange and Henze, 1988; Schmidt et al., 1990; Wintergerst et al., 1992].

Treatment options during relapse, however, must be carefully considered. The persistent immune activation observed in cases of HSE may result in a form of autoimmune disease. For example, in two cases of relapse occurring up to 2 months after treatment with vidarabine [Davis and McLaren, 1983; Dix et al., 1983], electron microscopy of brain biopsy material revealed no evidence of herpesvirus particles, whereas histopathological evidence was suggestive of extensive demyelinating disease. Corticosteroid therapy might be suggested as therapy in treatment of the latter form of encephalitic illness, the rationale being to reduce inflammation and consequent CNS oedema. During and following the acute stages of herpes encephalitis, there is evidence for intrathecal production of a variety of cytokines [Aurelius et al., 1994]. Cytokines are involved in the induction and modulation of inflammation, and as the action of corticosteroids in cytokine activation or modulation of activity is not fully understood, combination therapy with acyclovir and interferon in relapse might be expected to exacerbate rather than alleviate disease, particularly if the relapse were due to postinfectious disease.

The five cases described here did not relapse until at least 1 month following completion of the initial course of antiviral therapy. Although viral DNA was still detectable at day 13 (case 1), day 18 (case 2), and day 19 (case 5), no firm conclusions concerning efficacy of treatment in relation to detection of DNA and risk for relapse can be drawn from the present study. In a retrospective study of 55 patients with herpes encephalitis, five patients treated with acyclovir, in whom relapse was not observed, had HSV DNA detectable in the CSF at days 18, 19, 29, 34, and 56 after onset of neurological illness [Dennett, 1993]. A new episode of intrathecal immune activation was suggested by an increase in intrathecal IgG synthesis (raised IgG Index), but there was no direct evidence of further viral replication within the CNS. No HSV DNA was detected in CSF by PCR, and although the HSV-specific antibody index remained abnormal—indicating continued intrathecal production of HSV antibody—this did not increase during the second episode. During the second episode, myelin basic protein again became detectable in the CSF. It might therefore be suggested that these cases represent relapse due to postinfectious encephalitis. However, no brain biopsies were performed to establish such a diagnosis, and alternate explanations of these results must therefore be considered. A "falsely negative" PCR result could occur during relapse if only small amounts of viral DNA were present in lumbar CSF during the episode; thus the timing of sample collection may be critical to successful detection of virus replication during relapse. The PCR method utilised has been shown to be capable of detection of one copy of HSV genome in the presence of 2,500 uninfected cells [Klapper et al., 1990]. If relapse were due to antiviral resistance rather than incomplete therapy [Burns et al., 1982], the negative PCR results might be explained by a failure to detect the HSV TK gene target (the genomic target of our PCR). However, thymidine kinase deficient mutants of HSV are resistant to acyclovir as a result of point mutations within the thymidine kinase (TK) gene rather than complete gene deletion [Darby et al., 1986]. Thus even if a virus exhibits the TK -ve phenotype, PCR positive results may be expected [Dennett, 1993]. In addition, resistance to acyclovir or vidarabine has not as yet been reported in cases of relapse of HSE [Whitley, 1988b]. However, use of primer pairs directed to alternative targets on the HSV genome may be worthwhile in providing additional confirmation of these results.

At present, the detection of HSV DNA in lumbar CSF by PCR during a relapse following herpes encephalitis may facilitate the management of patients by providing an indication for continued antiviral therapy. However, a negative PCR result cannot be used absolutely to exclude a diagnosis of relapse due to continued viral activity within the CNS. Further work is needed to elaborate more exact discriminators between relapse due to continued viral activity and that due to immunological overactivity (postinfectious encephalopathy). In the interim, alteration of standard therapy (i.e., intravenous acyclovir therapy) to the use of combined interferon and acyclovir therapy and/or modulators of an immune response during a relapse of encephalitic illness warrants caution.

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